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PRINCIPAL INVESTIGATOR: Susan L. Neuhausen, Ph.D.

CONTRACTING ORGANIZATION: University of Utah
Salt Lake City, Utah 84132

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Susan L. Neuhausen, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Utah
Salt Lake City, Utah 84132

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Since the last annual report, the BRCA2 gene was cloned by our collaborator on this grant (Wooster et al., 1995). We identified the complete BRCA2 sequence which encodes a protein of 3,418 amino acids (Tavtigian et al., 1996). Due to the successful completion of several of the aims, we added additional goals. We have identified predisposing mutations in six of our families and a polymorphism in a seventh (Tavtigian et al., 1996). Dr. Stratton's group has identified 30 mutations in 33 families and also screened for BRCA2 mutations in primary ovarian and breast cancer tumors to identify somatic rather than germline mutations (Lancaster et al., 1996). We are identifying all mutation carriers within families in order to obtain more accurate estimates of penetrance and to determine the risks of other cancers. We are continuing to screen for mutations in our additional families using SSCP. Now that the gene has been isolated and mutations are being identified rapidly, we are constructing a database of haplotypes of mutations. In a collaborative project, we will examine common mutations to investigate mutation origin. We have already identified a BRCA2 mutation which is common in Ashkenazi Jews (Neuhausen et al., 1996; Oddux et al., 1996).

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Susan L. Neuhausen 9-12-96

PI - Signature Date

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Annual Progress Report
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Introduction

Breast cancer is a pervasive disease in the United States with approximately 182,000 cases diagnosed last year. A family history of breast cancer has been identified as a major risk factor for the development of breast cancer, with estimates that up to 10% of breast cancer is due to a genetic predisposition. The breast cancer genes, BRCA1 and BRCA2, likely account for more than 80% of inherited early-onset breast cancer. The primary goal of this project was to clone BRCA2 and that has been realized. We now are moving forward to characterize this gene and its importance in familial breast cancer.

Estimating the risks for specific BRCA2 mutations is beneficial for directed counseling of BRCA2 mutation carriers. By identifying and examining specific mutations, risks for breast and other cancers such as ovarian, prostate, and stomach cancers can be determined. We will continue to screen for BRCA2 mutations in our collection of kindreds and then analyze those mutations in these high-risk families. Age-specific penetrance can be evaluated after identifying all mutation carriers in our extended kindreds.

Body

Results from August 20, 1995 to August 21, 1996, as listed relative to the goals of the proposal.

1. Construction of fine-scale genetic and physical maps in the BRCA2 region. As part of our effort to isolate the BRCA2 gene, detailed genetic, physical, and transcription maps were generated (Couch et al., 1996). In addition to the eight new polymorphic, short tandem repeats (STRs) described in the last annual report, we generated an additional six STRs which will be utilized in the haplotype analysis as they more closely flank the BRCA2 gene.

2. Continue to ascertain families. We did not ascertain any additional large families as we had narrowed the region sufficiently that more families were not needed. However, we are extending families to identify additional mutation carriers.

3. Look for recombinants in BRCA2-linked families. We were able to identify key recombinants within our families to more closely localize the BRCA2 gene. Key recombinants are described in Couch et al. (1996).

A primary goal of this grant had been to develop the physical and genetic resources in order to clone the BRCA2 gene. Our collaborator on this grant, Mike Stratton, isolated the BRCA2 gene (Wooster et al., 1995). Utilizing our genetic data and the physical map, we were able to describe the entire BRCA2 gene (Tavtigian et al., 1996).

With the cloning of the gene, we expanded our objectives to include screening for BRCA2 mutations in our collection of kindreds (Aim 1 of revised SOW of 2-12-96). In the primary screening for mutations in the BRCA2 gene, we concentrated on the families with a high probability of breast cancer due to BRCA2. Of 12 Utah families and an additional 6 families of collaborators, we identified 8 predisposing breast cancer mutations and 9 polymorphisms (S. Tavtigian et al., 1996). We are continuing to screen for mutations in the remaining families and have identified the actual splice junction nucleotide substitution in K2367 (unpublished). Dr. Stratton's group has collected a series of 209 families ranging in size from two breast cancer cases diagnosed at less than 60 years of age up to 17 cases. They are currently screening for BRCA2

mutations in these families. Of the 33 families screened for BRCA2 mutations so far, 30 mutations have been identified (unpublished). In an additional four clearly-linked families, no mutation has been found. In these families, mutation rescreening is by SSCP, and when RNA is available, RT-PCR will be performed to look for loss of transcript.

Revised Aim 2. Estimations of age and site-specific penetrances.
In the previous report, we had enclosed a table estimating age-specific penetrance based on data from two large BRCA2-linked families. We expanded that analysis and examined data from BRCA2 carriers in 10 families, including the 2 families previously utilized. The ten families were K107, K2367, K2044, K2388, K2327, K2355, K1018, K2043, K2027, K2263, and K2265. Not all of these families have an identified BRCA2 mutation, but they all have a minimum of strong evidence of linkage to BRCA2. The majority of the data were from K107, K1018, and K2044 for which actual predisposing mutations have been identified.

Table 1. Penetrance of BRCA2 among 128 female BRCA2 carriers from 10 families.

Risk by Age	Breast Cancer	Ovarian Cancer
30	0.05	- -
40	0.25	0.01
50	0.47	0.05
60	0.53	0.12
70	0.60	0.12

The analysis of penetrance was performed using standard life-table estimates of gene carriers. With the additional data in this table, it is clear that the lifetime risk of breast cancer is lower in BRCA2 mutation carriers than in BRCA1 mutation carriers. The reason for this difference will not be understood until the biology of the two genes is known.

This penetrance analysis will be expanded by including the effects of risk factors such as age at first pregnancy, parity, etc.
In collaboration with the Breast Cancer Linkage Consortium, we are

examining site-specific risks in BRCA2 carriers. We had previously observed an excess of stomach cancer in a kindred linked to BRCA2 (Cannon-Albright et al., 1995). Preliminary data is suggestive that there are excesses of prostate cancer and ocular melanoma, and possibly excesses of pancreatic, colon, testis, and non-Hodgkins lymphoma cancers.

Aim 3 (2/12/96). Perform haplotype analysis to study the origin of the BRCA2 mutations. In the previous aims, Aim 5 was to construct haplotypes in families with breast cancer likely due to BRCA2 in order to identify possible shared mutations. With the identification of the gene, we can now examine common mutations in order to identify the age of the mutation. We are currently characterizing all of the STRs in the BRCA2 region in order to select those which will be most informative. We will establish a large, collaborative effort of families with the same mutations in order to identify the sources and ages of the mutations.

We observed a 6174delT mutation in an Ashkenazi Jewish woman. We then examined its frequency in a set of Ashkenazi Jewish women with breast cancer diagnosed under the age of 50 years and observed that 8% of the women carry this mutation. (Neuhausen et al., 1996). It has a prevalence of 1% in Ashkenazi Jews unselected for breast cancer (Oddux et al., 1996). We are constructing haplotypes in order to determine age of the mutation. Based on preliminary data of haplotypes for breast cancer cases with this mutation, there are at least two distinct haplotypes. We are trying to collect additional samples in order to more fully characterize the haplotype in those cases which carry the mutation. In addition, we are setting up a collaboration to explore other common BRCA2 mutations.

Conclusions

This past year has been very rewarding in that BRCA2 was identified and cloned. BRCA2 is a large protein of 3,418 amino acids and is dissimilar to BRCA1 (Tavtigian et al., 1996). Penetrance of the gene appears to be less than for BRCA1 which has implications for genetic counseling and testing of mutation carriers. It is believed to account for 30-40% of inherited early-onset breast cancer and in conjunction with BRCA1 for 80-90% of predisposing early-onset breast cancer. With the identification of these two breast cancer genes, scientists now may begin to elucidate the different pathways involved in the pathogenesis of breast cancer.

As described on the previous pages, we have accomplished many of the revised aims of this grant. Because of that, we again revised the aims on February 12th of this year. We are now able to turn our attentions more to the importance of the genotype-phenotype correlation for BRCA2.

During the next year, we will continue to work towards the goals of this grant. We will screen for BRCA2 in additional families likely due to BRCA2. In addition, we will screen a sample from each of our breast cancer kindreds which are not due to a known BRCA1 mutation for recurrent BRCA2 mutations. For our families with a known BRCA2 mutation, we will continue to extend the families to identify all known mutation carriers in order to obtain more precise estimates of penetrance and site-specific cancer risks. In collaboration with others in the breast cancer community, we will perform a haplotype analysis for common mutations in order to determine when the mutation arose. If enough data are available, we will also assess whether different mutations cause a different spectrum of cancer sites. Based on preliminary data, there is no correlation between position of the mutation and the presence of male breast cancer.

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